

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence encoding a first polypeptide of at least 60 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a histidine biosynthetic enzyme polypeptide of SEQ ID NOs:2, 4, 6, 8, 10, 12, and 14, or an isolated polynucleotide comprising the complement of the nucleotide sequence.
2. The isolated polynucleotide of Claim 1, wherein the isolated nucleotide sequence consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, and 14.
3. The isolated polynucleotide of Claim 1 wherein the nucleotide sequence is DNA.
4. The isolated polynucleotide of Claim 1 wherein the nucleotide sequence is RNA.
5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to suitable regulatory sequences.
6. An isolated host cell comprising the chimeric gene of Claim 5.
7. An isolated host cell comprising an isolated polynucleotide of Claim 1.
8. The isolated host cell of Claim 7 wherein the isolated host is selected from the group consisting of yeast, bacteria, plant, and virus.
9. A virus comprising the isolated polynucleotide of Claim 1.
10. A polypeptide of at least 60 amino acids that has at least 85% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a histidine biosynthetic enzyme polypeptide of SEQ ID NOs:2, 4, 6, 8, 10, 12, and 14.
11. A method of selecting an isolated polynucleotide that affects the level of expression of a histidine biosynthetic enzyme polypeptide in a plant cell, the method comprising the steps of:
- (a) constructing an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from an isolated polynucleotide of Claim 1;
- (b) introducing the isolated polynucleotide into a plant cell;
- (c) measuring the level of a glutamine amidotransferase polypeptide in the plant cell containing the polynucleotide; and
- (d) comparing the level of a glutamine amidotransferase polypeptide in the plant cell containing the isolated polynucleotide with the level of a glutamine amidotransferase polypeptide in a plant cell that does not contain the isolated polynucleotide.

2. The method of Claim 11 wherein the isolated polynucleotide consists of a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, and 14.

5 13. A method of selecting an isolated polynucleotide that affects the level of expression of a histidine biosynthetic enzyme polypeptide in a plant cell, the method comprising the steps of:

- (a) constructing an isolated polynucleotide of Claim 1;
- (b) introducing the isolated polynucleotide into a plant cell;

10 (c) measuring the level of a glutamine amidotransferase polypeptide in the plant cell containing the polynucleotide; and

(d) comparing the level of a glutamine amidotransferase polypeptide in the plant cell containing the isolated polynucleotide with the level of a glutamine amidotransferase polypeptide in a plant cell that does not contain the polynucleotide.

15 14. A method of obtaining a nucleic acid fragment encoding a histidine biosynthetic enzyme polypeptide comprising the steps of:

(a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 and the complement of such

20 nucleotide sequences; and

- (b) amplifying a nucleic acid sequence using the oligonucleotide primer.

15 15. A method of obtaining a nucleic acid fragment encoding the amino acid sequence encoding a histidine biosynthetic enzyme polypeptide comprising the steps of:

(a) probing a cDNA or genomic library with an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 and the complement of such nucleotide sequences;

- (b) identifying a DNA clone that hybridizes with the isolated polynucleotide;

- (c) isolating the identified DNA clone; and

30 (d) sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

16. A method for evaluating at least one compound for its ability to inhibit the activity of a histidine biosynthetic enzyme, the method comprising the steps of:

(a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a histidine biosynthetic enzyme, operably linked to suitable regulatory sequences;

(b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in

production of the histidine biosynthetic enzyme encoded by the operably linked nucleic acid fragment in the transformed host cell;

(c) optionally purifying the histidine biosynthetic enzyme expressed by the transformed host cell;

- 5 (d) treating the histidine biosynthetic enzyme with a compound to be tested; and
(e) comparing the activity of the histidine biosynthetic enzyme that has been treated with a test compound to the activity of an untreated histidine biosynthetic enzyme, thereby selecting compounds with potential for inhibitory activity.

17. A composition comprising an isolated polynucleotide of Claim 1.

10 18. A composition comprising an isolated polypeptide of Claim 10.

19. An isolated polynucleotide of Claim 1 comprising the nucleotide sequence comprising at least one of 30 contiguous nucleotides of a nucleic sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, and 13.

20. An expression cassette comprising an isolated polynucleotide of Claim 1 operably linked to a promoter.

21. A method for positive selection of a transformed cell comprising:

(a) transforming a plant cell with the chimeric gene of Claim 5 or an expression cassette of Claim 20; and

20 (b) growing the transformed plant cell under conditions allowing expression of the polynucleotide in an amount sufficient to complement a histidine biosynthetic auxotroph to provide a positive selection means.

22. The method of Claim 21 wherein the plant cell is a monocot.

23. The method of Claim 22 wherein the monocot is corn.

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